



Mdr1b facilitates p53-mediated cell death and p53 is required for *Mdr1b* upregulation *in vivo*

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The *mdr1b* gene is thought to be a "stress-responsive" gene, however it is unknown if this gene is regulated by p53 in the whole animal. Moreover, it is unknown if overexpression of *mdr1b* affects cell survival. The dependence of *mdr1b* upon p53 for upregulation was evaluated in p53 knockout mice. Wild-type (wt) or p53^{-/-} mice were treated singly or in combination with gamma irradiation (IR) and/or the potent DNA damaging agent, diethylnitrosamine (DEN). Both IR and DEN induced *mdr1b* in wild-type animals, but not in the p53^{-/-} mice. IR also upregulated endogenous *mdr1b* in the H35 liver cell line, and the *mdr1b* promoter was activated by IR and activation correlated with p53 levels; moreover activation required an intact p53 binding site. Colony survival studies revealed that co-transfection of both *mdr1b* and p53 dramatically reduced colony numbers compared to cells transfected with either p53 or *mdr1b* alone and cells microinjected with both *mdr1b* and p53 had a more dramatic loss in viability compared to cells injected with either expression vector alone. Further studies using acridine orange and ethidium bromide to measure apoptosis revealed that *mdr1b* caused apoptosis and this was enhanced by p53, however the increased apoptosis required a functional p53 transactivation domain. These studies indicate that *mdr1b* is a downstream target of p53 in the whole animal and expression of *mdr1b* facilitates p53-mediated cell death. *Oncogene* (2001) 20, 303–313.

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Introduction

Mammals possess plasma membrane ATP dependent transporters that can confer resistance to structurally diverse amphipathic cytotoxic agents (Gottesman and Pastan, 1993). While the human *MDR1* gene represents the prototype member, genes that are highly related have been cloned from rodents (Gottesman and Pastan, 1993). The rodent *mdr1a* and *mdr1b* genes

share greater than 88% identity, are often expressed in the same tissues and can be selectively overproduced during selection for resistance to cytotoxic agents (e.g., taxol, vinblastine, adriamycin (Lothstein *et al.*, 1989)). Further, selection for drug resistance with ectopically introduced *mdr1a* or *mdr1b* expression vectors yields similar drug resistance profiles for many drugs (Devault and Gros, 1990). In contrast, the application of cytotoxic drug selective pressure to murine macrophage cells caused a switch in endogenous *mdr* expression from predominantly *mdr1b* to *mdr1a* (Lothstein *et al.*, 1989; Lin *et al.*, 1995). The basis for the switch is a presumed transcriptional upregulation of *mdr1a*. Although, the mechanism accounting for this conversion is unknown, it may be hypothesized that high levels of *mdr1b* are deleterious to cell survival, a concept that is consistent with studies showing that cytotoxic drugs that are not *mdr1* substrates as well as oxidative stress induce *mdr1b* (Ziemann *et al.*, 1999; Thevenod *et al.*, 2000) and that transcriptional upregulation of *mdr1b* has been correlated with decreased viability (Schrenk *et al.*, 1996).

Drug sensitivity studies reveal, with respect to drug substrates, almost identical functions between *mdr1a* and *mdr1b* (Lothstein *et al.*, 1989). This redundancy in drug transport seems unnecessary, especially considering that both genes are co-expressed in many tissues, and in some cases at similar levels (e.g., liver, adrenal, colon and lung) (Schinkel *et al.*, 1995). These findings suggest that *mdr1a* and *mdr1b* have additional biological functions independent of drug transport. Indeed, recent studies revealed functional differences between *mdr1a* and *mdr1b* (Valverde *et al.*, 1996). In particular, *mdr1a* increases the rate of activation of swelling-activated chloride channels in response to hypo-osmotic conditions, thereby facilitating restoration of cell volume after exposure to hypo-osmotic stress (Valverde *et al.*, 1996) (human *MDR1* is *mdr1a*'s functional orthologue in this respect). In contrast, it has been reported that cells overexpressing *mdr1b* have an impaired ability to activate chloride channels in response to hypo-osmotic conditions (Bond *et al.*, 1998). Further studies by several groups have suggested that *MDR1* plays a role in apoptosis. For example, ectopic overexpression of *MDR1* diminishes the

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